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Relationship of Lactate Dehydrogenase Specificity and Growth Rate to Lactate Metabolism by *Selenomonas ruminantium*

C. C. SCHEIFINGER,[1] M. J. LATHAM,[2] AND M. J. WOLIN[3]*

Department of Dairy Science and Department of Microbiology, University of Illinois, Urbana, Illinois 61801

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A lactate-fermenting strain of *Selenomonas ruminantium* (HD4) and a lactate-nonfermenting strain (GA192) were examined with respect to the stereoisomers of lactate formed during glucose fermentation, the stereoisomers of lactate fermented by HD4, and the characteristics of the lactate dehydrogenases of the strains. GA192 formed L-lactate and HD4 formed L-lactate and small amounts of D-lactate from glucose. HD4 fermented L- but not D-lactate. Both strains contain nicotinamide adenine dinucleotide (NAD)-specific lactate dehydrogenases, and no NAD-independent lactate oxidation was detected. Continuous cultures of both strains grown with limiting glucose produced mainly propionate and acetate and little lactate at dilution rates less than 0.4/h, with shifts to increasing amounts of lactate and less acetate and propionate as the dilution rate was increased from 0.4/h to approximately 1/h.

The formation of propionate and acetate from carbohydrates is a major activity of *Selenomonas ruminantium* in its natural habitat, the rumen (3, 19). Lactate formation by *S. ruminantium* and other species, and subsequent conversion of lactate to acetate and propionate, can be an important feature of rumen fermentation when ruminants are fed high-grain diets (15). Strains of *S. ruminantium* vary, however, in the relative amounts of lactate, propionate, and acetate they produce during batch culture fermentation of glucose (3). Strains that grow on lactate instead of glucose as an energy source produce large amounts of propionate and acetate and small amounts of lactate from glucose, whereas strains that do not ferment lactate accumulate lactate as the major product of glucose fermentation.

Hobson and Summers showed that strains of *S. ruminantium* that were originally described as lactate-using strains produced different fermentation products from glucose, depending on their growth rate in glucose-limited continuous cultures (9, 10). At slow growth rates, short-chain volatile acids were the dominant products. As growth rate increased, formation of lactate increased and volatile acid production decreased. These results raise important questions. Why is lactate a significant product of glucose fermentation at high growth rates of a lactate-using strain, but only a minor product in batch culture? And, in continuous culture, do strains that cannot use lactate show the same changes in fermentation products with growth rate as do lactate-using strains? This report describes the results of experiments which compared the effects of growth rate on product formation from glucose by lactate-fermenting and -nonfermenting strains.

The products formed by *S. ruminantium* in the rumen ecosystem may depend not only on the ability of the strains in the population to ferment lactate and on their growth rates in the ecosystem, but also on other factors that influence the course of their carbohydrate fermentation. For example, lactate accumulates and then is used in a diauxic pattern during batch growth of a lactate-using strain grown with glucose (8), and nutritional requirements are different for growth on lactate than for growth on glucose (14). Differences in the ability of strains to use lactate and the diauxic pattern of lactate production and utilization suggest the possibility of different enzymatic mechanisms for formation and utilization of lactate. Differences in formation and utilization could be based on differences in the isomeric forms of lactate produced and utilized and differences in the nature of the enzymes responsible for lactate production and utili-

[1] Present address: Smith, Kline Corp., 1600 Paoli Pike, West Chester, Pa. 19380.

[2] Present address: National Institute for Research in Dairying, Shinfield, Reading RG2, 9AT, England.

[3] Present address: Environmental Health Center, Division of Laboratories and Research, New York State Department of Health, Albany, N.Y. 12201.

zation. London showed that *Streptococcus faecium* formed lactate from glucose with a pyridine nucleotide-dependent lactic dehydrogenase (LDH) and oxidized lactate with a catabolite-repressed, pyridine nucleotide-independent lactate oxidase (16). In this report we compare the LDHs of *S. ruminantium* strains that do and do not ferment lactate, identify the stereoisomers of lactate they produce, and determine the stereoisomers of lactate used by a lactate-fermenting strain.

MATERIALS AND METHODS

Organisms and growth conditions. *S. ruminantium* strains HD4, HD1, GA192, and PC18 were obtained from the culture collection of the Department of Dairy Science, University of Illinois. All incubations were at 37 C, with an initial gas phase of 100% CO_2. The Hungate technique for media preparation and cultivation of anaerobes as modified by Bryant (5) was used throughout. Stock cultures were grown on a complex medium containing 0.1% each of glucose, cellobiose, and starch and 1.5% agar. The basal complex medium contained per liter: Trypticase (BBL), 5.0 g; yeast extract (Difco), 1.0 g; K_2HPO_4, KH_2PO_4, and $(NH_4)_2SO_4$, 0.48 g each; NaCl, 0.96 g; $MgSO_4 \cdot 7H_2O$, 0.20 g; $CaCl_2 \cdot 2H_2O$, 0.13 g; resazurin, 1.0 mg; clarified rumen fluid, 200 ml; Na_2CO_3, 4.0 g; and cysteine·hydrochloride, 0.5 g. For the preparation of cell-free extracts, organisms were grown in a 3-liter, round-bottom flask containing 2 liters of basal complex medium, with either 0.5% glucose or 1.8% sodium DL-lactate as the energy source. The inoculum, 10 ml of an overnight culture grown in the same medium, was injected through the rubber stopper used to close the flask. A 10-ml syringe fitted with a 20-gauge, 1.5-inch (3.8-cm) needle was used for injection.

A synthetic medium was used for the determination of the isomeric form of lactic acid produced from the fermentation of glucose. The medium contained per liter: glucose, 0.5 g; K_2HPO_4, KH_2PO_4, and $(NH_4)_2SO_4$, 0.48 g each; NaCl, 0.96 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.13 g; dithiothreitol, 0.54 g; resazurin, 1.0 mg; vitamin stock, 10.0 ml; volatile fatty acid stock, 1.0 ml; Na_2CO_3, 4.0 g; and cysteine·hydrochloride, 0.5 g. The vitamin stock contained per 100 ml: thiamin·hydrochloride, calcium D-pantothenate, nicotinamide, riboflavin, and pyridoxine·hydrochloride, 20.0 mg each; biotin, 10.0 mg; *p*-aminobenzoic acid, 1.0 mg; folic acid, 0.5 mg; and cyanocobalamin, 0.2 mg. The volatile fatty acid stock solution contained: *n*-butyric acid, 10.6 ml; isobutyric acid, 1.8 ml; and 2-methylbutyric acid, *n*-valeric acid, and isovaleric acid, 2.0 ml each.

Continuous culture studies were conducted with a slightly modified basal complex medium. Rumen fluid was omitted, and 0.05 ml each of *n*-butyric and *n*-valeric acids, 2.5 mg of biotin, and 1.0 g of glucose were added per liter. Mineral concentrations differed from the basal complex medium as follows (grams per liter): K_2HPO_4, 0.45; KH_2PO_4, 0.45; $(NH_4)_2SO_4$, 0.45; and NaCl, 0.90. The continuous-culture apparatus and relevant operating procedures have been previously described (13). After inoculation of the growth vessel with a 24-h culture, cells were grown batchwise before medium flow was initiated. Samples were not collected for analysis after adjustment of the dilution rate until at least three times the culture volume (350 ml) flowed through the vessel and the optical density had become stable. A minimum of four samples were taken at each dilution rate for analysis of fermentation products and residual glucose.

Fermentation product analyses. Batch culture fermentation acids were analyzed by silicic acid chromatography as previously described (19), and samples from continuous cultures were examined for acetic and propionic acid by gas-liquid chromatography as previously described (12) and for lactic acid by the method of Barker and Summerson (2). Glucose was determined by the glucose oxidase method of the Sigma Chemical Co. Stereoisomers of lactic acid were measured with rabbit muscle L-lactate LDH and *Lactobacillus casei* D-lactate LDH according to the method of Hohorst (11). The LDHs were obtained from Boehringer-Mannheim Biochemicals, New York.

Cell-free studies. Cells were collected by centrifugation in capped centrifuge tubes under an atmosphere of CO_2 at 13,000 × *g* for 10 min at 0 C in a Sorvall RC2B centrifuge. The cells were resuspended in five times their volume of a mineral salts buffer, which contained per liter: K_2HPO_4, 0.48 g; H_2PO_4, 0.48 g; $(NH_4)_2SO_4$, 0.96 g; $MgSO_4 \cdot 7H_2O$, 0.20 g; $CaCl_2 \cdot 2H_2O$, 0.13 g; dithiothreitol, 0.54 g; and resazurin, 1.00 mg. After recentrifugation as described above, the washed cells were resuspended in the same buffer (2.5 g [wet weight] of cells per 10 ml of buffer), gassed with CO_2, and frozen at −80 C. Forty milliliters of thawed cell suspensions was then centrifuged at 12,000 × *g* for 10 min at 0 C and resuspended in 40 ml of 0.1 M tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride buffer, pH 8.0, and disrupted with maximal power on a Branson sonifier (model S-75; Heat Systems Ultrasonics Inc., Plainview, N.J.). The suspensions were cooled in an ice bath and sonicated for a total of 4 min, with a 1-min interval of cooling after each 1-min period of sonication. The degree of breakage was determined by direct microscopic observation. After disruption, the suspension was clarified by centrifugation at 15,000 × *g* for 30 min at 0 C. The resulting supernatant, containing 10 to 12 mg of protein per ml, was stored at −80 C under CO_2 until used, generally within 1 to 2 days. Protein was determined by the method of Lowry et al. (17). The extracts used for nicotinamide adenine dinucleotide (NAD)-independent LDH assays were either dialyzed overnight (10 ml of sample against two 500-ml changes of 0.05 M Tris-hydrochloride buffer, pH 7.5) or placed on a 3- by 1.5-cm column of Sephadex G-15 equilibrated and eluted with an equal volume of 0.1 M Tris-hydrochloride buffer, pH 7.5.

NAD-linked LDH was assayed in the direction of NAD reduction with a 2-ml reaction mixture containing: 50 mM Tris-hydrochloride, pH 8.5; 0.3 mM

NAD^+; 10 mM L-, D-, or sodium DL-lactate; and extract (90 to 180 μg of protein). The reaction was at 25 C and was initiated by the addition of either the extract or lactate. The increase in absorbancy at 340 nm was determined with a Gilford recording spectrophotometer. Specific activity after correction for a control reaction mixture without lactate was expressed as micromoles of reduced NAD (NADH) formed per minute per milligram of protein. The enzyme was also assayed in the direction of NADH oxidation with a 2-ml reaction mixture containing: 50 mM Tris-hydrochloride, pH 7.0; 0.3 mM NADH; 10 mM sodium pyruvate; and extract (90 to 180 μg of protein). The reaction was at 25 C, and the decrease in absorbancy at 340 nm was followed. Specific activity was expressed as micromoles of NADH oxidized per minute per milligram of protein.

NAD-independent LDH was estimated using 2,6-dichlorophenolindophenol (DCIP) as the electron acceptor. The 2-ml reaction mixture contained: 60 mM Tris-hydrochloride, pH 7.0; 0.09 mM DCIP; 10 mM DL-, L-, or sodium L-lactate; and approximately 1 mg of protein. The reaction was at 25 C, was initiated by the addition of either the extract or lactate, and was compared to a control without lactate. Activity was estimated from the rate of DCIP reduction, measured at 600 nm, in the first 20 s after initiation of the reaction. Specific activity was expressed as micromoles of DCIP reduced per minute per milligram of protein.

RESULTS

Products of glucose fermentation by strain HD4. Hishinuma et al. (8) reported that batch fermentation of glucose by lactate-fermenting strains of *S. ruminantium* follows a diauxic pattern. As glucose disappears, lactate accumulates; then the lactate is utilized to form propionate and acetate. We confirmed this general pattern with the HD4 strain (Fig. 1). Since differences in the rate of formation and utilization of lactate might be related to the stereoisomer of lactate produced and used, experiments were performed to identify the stereoisomers.

Stereoisomers formed and used by S. ruminantium. It has been reported that some strains (undesignated) of *S. ruminantium* form DL-lactate (4). In our hands, however, *S. ruminantium* GA192 formed only L-lactate and *S. ruminantium* HD4 formed mainly L-lactate, with small amounts of D-lactate, when the strains were grown on glucose (Table 1). The possibility that a large amount of D-lactate was produced by the lactate-fermenting HD4 strain and then disappeared because of utilization is ruled out by the results of a growth experiment that showed that the two lactate-fermenting strains, HD4 and PC18, used L- but not D-lactate as a substrate for growth. Strains GA192 and HD1 did not grow with L-, D-, or DL-lactate.

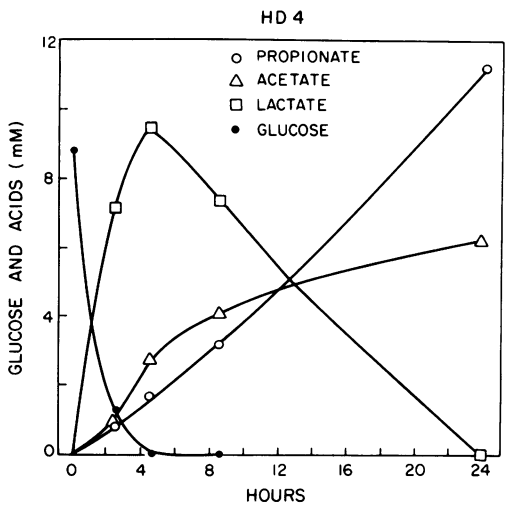

FIG. 1. *Product accumulation by S. ruminantium HD4. Growth was in 50 ml of complex broth with 0.1% glucose in a 100-ml round-bottom growth flask under an atmosphere of CO_2. Incubation was at 37 C on a shaker at 12 strokes/min. At the indicated sampling times, a 5-ml syringe fitted with a 25-gauge, 1.5-inch needle was inserted through the rubber stopper, and a 5-ml sample was removed for analyses of acids and substrate.*

TABLE 1. *Isomers of lactate produced by strains of S. ruminantium*[a]

Strain	Time (h)	Lactate (mM)	
		L	D
HD4	5	7 6	0.7
	24	6.9	0.6
GA192	5	7.8	0.0
	24	12.6	0.0

[a] Cultures were grown in 0.5% glucose synthetic medium (10 ml per 18- by 150-mm tube) at 37 C under CO_2 on a shaker at 120 strokes/min. At 5 and 24 h, a syringe fitted with a 25-gauge, 1.5-inch needle was used to remove a 3-ml sample through the rubber stopper. The samples were centrifuged at 13,000 × *g* to remove the cells. The supernatants were assayed with stereospecific LDHs.

Lactate dehydrogenase of S. ruminantium. The lactate-using strain HD4 and the lactate-nonfermenting strain GA192 were examined for pyridine nucleotide-dependent and -independent LDHs. Activities were examined in cells at various stages in the growth cycle and, in the case of HD4, with cells grown on glucose or lactate. No oxidation was observed with D-lactate (Table 2). With L-lactate the specific activities for lactate oxidation were approximately the same under all growth conditions, except for the lower activity of HD4 harvested

TABLE 2. *Oxidative mode of NAD-linked LDH activity in S. ruminantium*

Strain	Growth medium	Harvest phase	Isomer	Sp act[a]
HD4	Sodium DL-lactate (1.8%)	Late log	L	0.884
			D	0.000
	Glucose (0.5%)	Mid-log[b]	L	0.658
			D	0.000
		Late stationary[c]	L	0.402
			D	0.000
GA192	Glucose (0.5%)	Mid-log[b]	L	0.781
			D	0.000
		Late stationary[c]	L	0.686
			D	0.000

[a] Micromoles of NAD reduced per minute per milligram of protein. The assay measured NAD reduction in the presence of lactate.
[b] Glucose remaining.
[c] Glucose depleted.

in late stationary phase. No activity was observed when NAD phosphate (NADP) was substituted for NAD with either D- or L-lactate.

The activities of the LDHs of both strains grown with 0.5% glucose to mid-log phase were also similar when assayed in the direction of pyruvate reduction, with specific activities of approximately 6 μmol of NADH oxidized per minute per milligram of protein. The greater specific activity for pyruvate reduction than for lactate oxidation reflects the equilibrium of the LDH reaction, which strongly favors the formation of lactate rather than its oxidation. Similar K_m values for L-lactate of both enzymes were estimated from Lineweaver-Burk plots (1/v versus 1/s): GA192, 1.1×10^{-4} M; HD4, 1.4×10^{-4} M.

Little or no DCIP reduction was obtained in the absence of NAD (Table 3), indicating that NAD nonlinked lactate oxidases are not present in HD4 and GA192. DCIP was reduced with L- but not with D-lactate (Table 3). Enhancement of the NAD-dependent rate by phenazine methosulfate suggests poor enzymatic coupling between NADH and DCIP. Phenazine methosulfate probably oxidized NADH non-enzymatically, and reduced phenazine methosulfate non-enzymatically reduced DCIP. There were only minor differences in the specific activities of the HD4 and GA192 strains grown on glucose and the HD4 strain grown on lactate.

Continuous culture studies. Strains HD4 (lactate fermenting) and GA192 (lactate nonfermenting) were each grown independently in continuous culture in a glucose-limited medium at dilution rates increasing from 0.15 to 1.0/h. No residual glucose was detected in either culture at any dilution rate. Propionic and acetic acids were the predominant products of both strains at low dilution rates, but as the dilution rate increased the production of these acids decreased, with lactate becoming the major fermentation product (Fig. 2 and 3).

Similar patterns of change in fermentation products as a function of increasing growth rate were obtained with the GA192 (Fig. 2) and the HD4 (Fig. 3) strains, and the shift from volatile acid to lactate production occurred at approximately the same dilution rate (approximately 0.4/h) with each strain. There was, however, a significant difference between strains in the rate of accumulation of lactate as growth rate was increased. GA192 produced more lactate at all dilution rates, and at the highest dilution rate (1/h) it had shifted to an essentially homolactic fermentation, whereas HD4 was

TABLE 3. *Non-pyridine nucleotide-linked LDH activity in S. ruminantium*[a]

Strain	Lactate	Sp act[b]			
		− PMS		+ PMS	
		+ NAD	− NAD	+ NAD	− NAD
HD4[c]	DL	27	4	54	7
HD4[d]	L	9	4	85	2
	D	0	0	0	0
GA192[d]	L	15	2	143	2
	D	0	0	0	0

[a] The cell extract was passed through a column of Sephadex G-15 to remove interfering nucleotides before analysis. Assay concentrations of DCIP were: 0.09 mM DCIP, 0.45 mM phenazine methosulfate (PMS), and 0.3 mM NAD.
[b] Expressed as 10^{-3} μmol of DCIP reduced/min per mg of protein.
[c] Sodium DL-lactate (1.8%) served as the energy source. Cells were harvested at late log phase.
[d] Glucose (0.5%) served as the energy source. Cells were harvested at mid-log phase (glucose remaining).

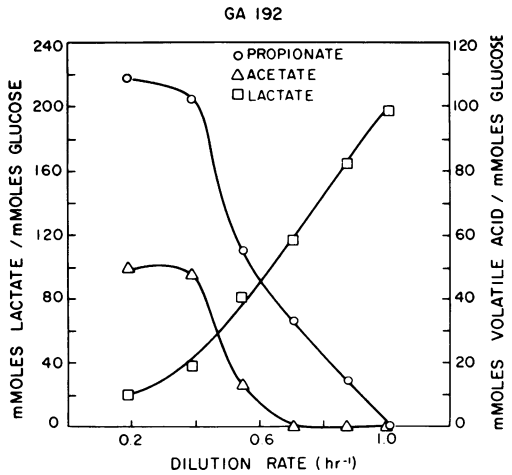

FIG. 2. *Effect of dilution rate on propionate, acetate, and lactate production by S. ruminantium GA192.*

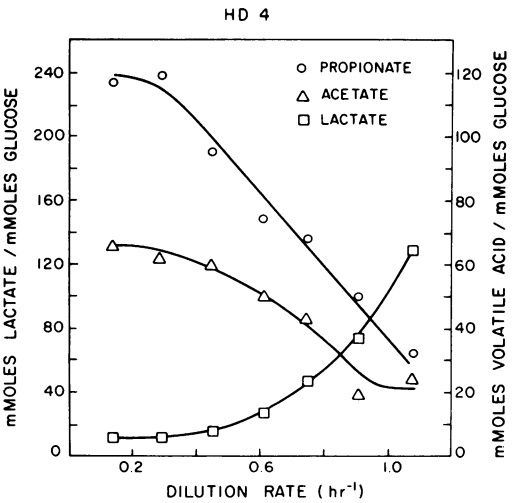

FIG. 3. *Effect of dilution rate on propionate, acetate, and lactate production by S. ruminantium HD4.*

still producing significant amounts of volatile acids.

DISCUSSION

Both the lactate-fermenting and -nonfermenting strains of *S. ruminantium* used in this study formed L-lactate but not DL-lactate during fermentation of glucose. It has similarly been reported that *S. ruminantium* strain GFA forms only L-lactate, and not D-lactate (7). We have no explanation for the discrepancy between our results and the previous report (3) of DL-lactate production. The significance of the small amount of D-lactate formed by strain HD4 is also unclear. The possibility that the LDH used for the determination oxidizes a product other than D-lactate or is contaminated with an enzyme that oxidizes some other product cannot be ruled out. It is clear, however, that both of the lactate-fermenting strains, HD4 and PC18, ferment L- but not D-lactate. Enzymatic analysis of LDH specificity and activity indicates, therefore, that both lactate formation and lactate utilization by HD4 are catalyzed by an NAD-specific, L-lactate-specific enzyme.

There is no evidence for a distinction between lactate formation and utilization, which would involve different LDHs with different specificities. Consequently, there appears to be no analogy between the *S. ruminantium* HD4 system and the *Streptococcus faecium* system, where lactate formation is mediated by a constitutive NAD-linked LDH and lactate utilization is mediated by an inducible, pyridine nucleotide-independent lactate oxidase (16).

We have not ruled out the possibility of the involvement of a lactate-malate transhydrogenase in lactate utilization. This enzyme is essential for lactate fermentation by *Veillonella* because these organisms lack a conventional LDH (1, 18). However, it would not seem to be necessary for *S. ruminantium* HD4, which contains a highly active NAD-linked LDH. The inability of GA192 to ferment lactate as opposed to HD4 cannot be related to any obvious differences in the way the strains interconvert lactate and pyruvate.

The change in glucose fermentation products observed with increasing dilution rate were similar to those observed by Hobson and Summers (9, 10). These authors used strains of *S. ruminantium* that had originally been described as fermenting lactate and glycerol. Their reports stated, however, that the initial glycerol-fermenting ability of the strains had been lost, and it is not clear whether the ability to ferment lactate was retested. The present study shows unequivocally that the accumulation of acetate and propionate at slow growth rates and the shift to increasing accumulation of lactate at faster growth rates occurs with both lactate-fermenting and -nonfermenting strains. If accumulation were related to utilization, GA192 should have accumulated large amounts of lactate at all growth rates.

A more likely explanation is that small amounts of lactate are formed by both strains at slow growth rates, and, as growth rate increases, some change takes place within the cells which causes increased lactate production. Accumulation of less lactate in HD4 compared with GA192 probably results from lactate utilization by HD4 once increased lactate formation

is initiated. The rate of utilization is probably too slow to prevent net accumulation of some lactate.

Although the events that trigger lactate formation are unknown, it is of interest that they appear to occur at approximately the same dilution rate with both strains, 0.4/h. Changes in the intracellular concentrations of components that might regulate LDH activity, or changes in pool levels of pyruvate, could regulate the flow of pyruvate carbon to propionate and acetate versus the alternative flow to lactate. Intracellular concentrations of fructose-1,6-diphosphate have been implicated in the shift of the fermentation products of glucose by *Lactobacillus casei* from acetate, ethanol, and formate at slow growth rates to lactate at fast growth rates (6). The *L. casei* LDH requires fructose-1,6-diphosphate for activity (6). In the present study, no indication of such a requirement was obtained with untreated, dialyzed, or Sephadex G-15-chromatographed extracts.

The factors that control lactate formation and its subsequent utilization by strains of *S. ruminantium* are pertinent to understanding the role of this species in the rumen. Slow growth rates may predominate when cellulose is the main source of carbohydrate in the ruminant diet and when *S. ruminantium* depends on the hydrolysis products of cellulose formed by cellulolytic species for its major source of carbon and energy (19). Propionate, acetate, and CO_2 would then be the major fermentation products. Since some strains of *S. ruminantium* ferment starch and since the products of starch hydrolysis may be formed and made available more rapidly to starch-negative strains, lactate formation might be enhanced with starch-containing diets. Lactate formed by *S. ruminantium* and other rumen species can then be converted to propionate, acetate, and CO_2 by strains such as HD4.

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LITERATURE CITED

1. Allen, S. H. G. 1966. The isolation and characterization of malate-lactate transhydrogenase from *Micrococcus lactilyticus*. J. Biol. Chem. 241:5266–5275.
2. Barker, S. B., and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535–554.
3. Bryant, M. P. 1956. The characteristics of strains of *Selenomonas* isolated from bovine rumen contents. J. Bacteriol. 72:162–167.
4. Bryant, M. P. 1959. Bacterial species of the rumen. Bacteriol. Rev. 23:125–153.
5. Bryant, M. P. 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. 25:1324–1328.
6. De Vries, W., W. M. C. Kapteijn, E. G. van der Beek, and A. H. Stouthamer. 1970. Molar growth yields and fermentation balances of *Lactobacillus casei* L3 in batch cultures and in continuous cultures. J. Gen. Microbiol. 63:333–345.
7. De Vries, W., M. C. van Wijck-Kapteyn, and S. K. H. Oosterhuis. 1974. The presence and function of cytochromes in *Selenomonas ruminantium, Anaerovibrio lipolytica* and *Veillonella alcalescens*. J. Gen. Microbiol. 81:69–78.
8. Hishinuma, F., S. Kanegasaki, and H. Takahashi. 1968. Ruminal fermentation and sugar concentrations: a model experiment with *Selenomonas ruminantium*. Agric. Biol. Chem. 32:1325–1330.
9. Hobson, P. N., and R. Summers. 1967. The continuous culture of anaerobic bacteria. J. Gen. Microbiol. 47: 53–65.
10. Hobson, P. N., and R. Summers. 1972. ATP pool and growth yield in *Selenomonas ruminantium*. J. Gen. Microbiol. 79:351–360.
11. Hohorst, H. 1965. L-(+)-lactate determination with lactic dehydrogenase and DPN, p. 266–277. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. Academic Press Inc., New York.
12. Iannotti, E. L., D. Kafkewitz, M. J. Wolin, and M. P. Bryant. 1973. Glucose fermentation products of *Ruminococcus albus* grown in continuous culture with *Vibrio succinogenes*: changes caused by interspecies transfer of H_2. J. Bacteriol. 114:1231–1240.
13. Kafkewitz, D., E. L. Iannotti, M. J. Wolin, and M. P. Bryant. 1973. An anaerobic chemostat that permits the collection and measurement of fermentation gases. Appl. Microbiol. 25:612–614.
14. Kanegasaki, S., and H. Takahashi. 1967. Function of growth factors for rumen microorganisms: nutritional characteristics of *Selenomonas ruminantium*. J. Bacteriol. 93:456–463.
15. Latham, M. J., E. Sharpe, and J. D. Sutton. 1971. The microbial flora of the rumen of cows fed hay and high cereal rations and its relationship to the rumen fermentation. J. Appl. Bacteriol. 34:425–434.
16. London, J. 1968. Regulation and function of lactate oxidation in *Streptococcus faecium*. J. Bacteriol. 95: 1380–1387.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
18. Ng, S. K. C., and I. R. Hamilton. 1971. Lactate metabolism by *Veillonella parvula*. J. Bacteriol. 105: 999–1005.
19. Scheifinger, C. C., and M. J. Wolin. 1973. Propionate formation from cellulose and soluble sugars by combined cultures of *Bacteroides succinogenes* and *Selenomonas ruminantium*. Appl. Microbiol. 27:789–795.